

Pollen morphology in tribe Dicomeae Panero and Funk (Asteraceae)

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Abstract To better understand the taxonomy and phylogeny of the Dicomeae (Asteraceae) the pollen morphology of seven genera including 15 species of that tribe and six genera with seven species belonging to five related tribes was studied by use of light and scanning electron microscopy. The quantitative data were analysed by use of principal-components analysis (PCA). The exine ultrastructure of *Erythrocephalum longifolium* and *Pleiotaxis rugosa* was also studied by use of transmission electron microscopy. Three pollen types were distinguishable from the apertural, columellar, and spinular morphology and inter-spinular sculpture. A dichotomous key to these pollen types is proposed. The existence of the Dicomeae as an independent tribe and its subdivision in two subtribes (Dicominae and Pleiotaxinae) are supported by this study, which also suggests the Oldenburgieae and the Tarchonantheae are the closest tribes to Dicomeae.

Keywords Dicomeae · Pollen morphology · Asteraceae · Taxonomy

Introduction

The tribe Dicomeae was firstly described by Panero and Funk (2002), and includes eight genera and 95 species of perennial herbs, shrubs, or small trees, mainly with an African and Malagasy distribution, though one species occurs in the Arabian Peninsula and another in India and Pakistan (Ortiz 2000; Ortiz et al. 2009). This taxon comprises most of the African genera previously included in the Mutisieae by authors such as Hoffmann (1890), Jeffrey (1967), Cabrera (1977) and Bremer (1994).

Morphological phylogenetic analysis by Ortiz (2000), ITS (rDNA) and *ndhF* (cpDNA) analysis by Ortiz et al. (2009), and *ndhF*+trnL-F (cpDNA) analysis by Ortiz et al. (unpublished) revealed that the genera *Cloiselia*, *Dicoma*, “*Dicomopsis*” (unpublished), *Macledium*, and *Pasaccardoa* belong to a monophyletic group (*Dicoma* s. l.) and seem closely related to each other. The other three genera (*Erythrocephalum*, *Gladiopappus*, and *Pleiotaxis*) are quite dissimilar from the taxa of that clade. No sequence data of *Gladiopappus* are yet available but *Erythrocephalum* and *Pleiotaxis* constitute a separate monophyletic group and a distinct subtribe (Pleiotaxinae—Ortiz et al. unpublished) or even a new tribe of Asteraceae (Ortiz et al. 2009). According to Ortiz et al. (unpublished), the other genera of Dicomeae belong to subtribe Dicominae.

According to many authors (Skvarla and Larson 1965; Skvarla et al. 1977, 2005; Bolick 1978, 1991; Zavada and De Villiers 2000; Tellería and Katinas 2005; Pereira Coutinho and Dinis 2007; Wortley et al. 2008) a knowledge of pollen morphology is important to understanding the taxonomy, phylogeny, and ecology of the Asteraceae. For this reason we investigated, by use of light and scanning electron microscopy, pollen morphology in seven genera (*Cloiselia*, *Dicoma*, “*Dicomopsis*”, *Erythrocephalum*, *Macledium*,

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Pasaccardoa, and *Pleiotaxis*) and 15 species (*Cloiselia carbonaria* S. Moore, *C. olaeifolia* (Humbert) S. Ortiz, *Dicoma anomala* Sond., *D. tomentosa* Cass., “*Dicomopsis*” *welwitschii*, *Erythrocephalum longifolium* Benth. ex Oliv. (= *E. zambesianum* Oliv. & Hiern.), *E. marginatum* (O. Hoffm.) S. Ortiz & A. P. Cout., *E. microcephalum* Dandy, *Macledium latifolium* (DC.) S. Ortiz, *M. sessiliflorum* (Harv.) S. Ortiz, *M. spinosum* (Harv.) S. Ortiz, *Pasaccardoa baumii* O. Hoffm., *P. jeffreyi* Wild, *Pleiotaxis rugosa* O. Hoffm., *P. subpaniculata* Chiov.) of Dicomeae. The specimens studied are listed in “Appendix”. The exine of *Erythrocephalum longifolium* and *Pleiotaxis rugosa* was also studied by use of transmission electron microscopy (TEM). These taxa were chosen because they represent all the clades of that tribe in the cladograms of Ortiz (2000) and Ortiz et al. (2009). To compare the pollen morphology of the Dicomeae with that of related tribes, the pollen grains of some Barnadesieae (Barnadesioideae—*Chuquiraga jussieu* J. F. Gmel. and *C. oppositifolia* D. Don), Oldenburgieae (*Oldenburgia paradoxa* Less.), Stifftieae (*Gongylolepis* sp.), Tarchonantheae (*Brachylaena discolor* DC. and *Tarchonanthus camphoratus* L.), and Wunderlichieae (*Stenopadus* sp.) were also studied, as outgroups, by use of scanning electron microscopy (SEM) and light microscopy (LM).

The palynology of those outgroup taxa was studied by Parra and Marticorena (1972, *Chuquiraga*—LM), Urtubey and Tellería (1998, *Chuquiraga*—LM, SEM), Tellería (2008, *Gongylolepis*, *Stenopadus*—LM, SEM), and Ubi-ergo et al. (2009, *Gongylolepis*—LM, SEM). The pollen morphology of the Dicomeae was studied by use of LM by Stix (1960) and Wodehouse (1929), by use of SEM by Hansen (1990), by use of SEM and LM by Perveen (1999), by use of SEM and TEM by Blackmore et al. (2010), and by use of SEM, TEM and LM by Ortiz and Pereira Coutinho (2001). Despite these contributions, however, the palynology of this tribe is still insufficiently known. Therefore we decided to contribute to a better understanding of the taxonomy of this tribe by studying the pollen morphology of a significant sample of its taxa.

Materials and methods

Pollen grains were collected from herbarium vouchers held at the British Museum of Natural History (BM), Royal Botanic Gardens of Kew (K), South African National Botanical Institute (PRE), Swedish Museum of Natural History (S), Royal Botanic Garden of Madrid (MA), and University of Coimbra (COI) and then acetolysed in accordance with Erdtman (1960). Specimens examined and voucher data are given at the end of the paper. The terminology used for description pollen follows Punt et al. (2007), Blackmore et al. (2009), and Hesse et al. (2009).

Light microscopy

Pollen grains were mounted in silicone oil and observed with a Motic BA 310 using a Stenoth $\times 100$ oil-immersion objective. Each of the characteristics length of polar axis (P) and length of equatorial diameter (E), both in meridian optical section (mos), the number of spines/100 μm^2 of the mesocolpium, spine length and width at the base (except for *Chuquiraga* and *Stenopadus*), width of the endoaperture, and exine thickness at the poles was measured for 30 pollen grains. The ratios P/E , spine height/spine width, and $E/\text{exine thickness}$ were then established.

Scanning electron microscopy

After acetolysis, pollen grains were treated with ultrasound (35 kc/s, 1 h) to increase the number of fractures, and dehydrated in a graded acetone series (70–100 %). They were then mounted on aluminium stubs and sputter coated with a 30-nm layer of gold–palladium before examination with a Jeol JSM-5400 at 10 kV. By using micrographs of the fractured grains we measured the thicknesses of the endexine, foot layer, internal tectum, and tectum, the height of the supporting and outer columellae, and the diameter of both the spinular and inter-spinular perforations. We also measured the spine length and spine width at the base of the pollen grains of *Chuquiraga* and *Stenopadus*.

Transmission electron microscopy

Pollen grains of *Erythrocephalum longifolium* and *Pleiotaxis rugosa* were fixed in osmium tetroxide and embedded in Spurr’s resin as described elsewhere (Pereira Coutinho and Dinis 2007). Thin sections were cut with an LKB Ultratome Nova ultramicrotome equipped with a diamond knife, conventionally stained with uranyl acetate and lead citrate, and observed with a Jeol JEM-100 SX at 80 kV. By using micrographs of the exine of those species, we measured the thicknesses of the endexine, foot layer, internal tectum, and tectum, the height and width of the supporting and outer columellae, and the diameter of both the spinular and inter-spinular perforations.

Data treatment and statistical analysis

The arithmetic mean, standard deviation, maximum, and minimum were calculated for all the measured pollen characters and ratios. Indirect gradient analysis was used to identify specimen assemblages on the basis of several morphological and anatomical quantitative traits. Preliminary detrended correspondence analysis (DCA) of the data-set, with detrending by segments and non-linear rescaling (Legendre and Legendre 1998), provided an

estimate of the underlying gradient length. Because the gradient length obtained was short (less than approx. 2.5 SD), the assemblage variation is within a relatively narrow range, and the linear approach of principal-components analysis (PCA) is appropriate. Original data were log transformed before analysis of the nature of the selected traits (i.e. the magnitude of the respective values). All indirect gradient analyses were performed in Canoco 4.5 for Windows.

Results

General description of the pollen morphology of Dicomeae

Pollen radially symmetrical and isopolar, suboblate or prolate, $P/E = 0.87\text{--}2.00$ (1.12 ± 0.12), subcircular or elliptical in mos (Fig. 1g), triangular-circular to circular in equatorial optical section (eos). $P = 33.70\text{--}75.00$ (50.60 ± 8.30) μm , $E = 21.00\text{--}68.00$ (46.00 ± 8.00) μm . E (average)/exine thickness at the poles (average) = 6.13. Trizonocolporate, with apertures constituted by an ecto, a meso, and an endo-aperture (Figs. 1a–d, f, i, j, 2a, e, 3a, b); ectoapertures—colpi elongated, acute at the ends (Figs. 1c, f, 2d, f, g); mesoapertures lolongate (Fig. 1d, i, j) or lalongate (Fig. 1a–c, f), elliptical, endoapertures lalongate, generally spindle-shaped and acute at the ends (Fig. 1a–c, f, i), sometimes narrowed at the centre and semi-circular at the ends (“butterfly”-shaped, Fig. 1d), width = $5.90\text{--}23.00$ (17.30 ± 5.90) μm ; colpal membranes psilate or scabrate (Figs. 2a, d, 3a, b); costae conspicuous (Fig. 1g). Exine tectate, echinate, acaveate (Figs. 1h, 2b, c, i, 3e, f, 4a, b), with an anthemoid structure (Fig. 4a, b); thickness at the poles = $3.90\text{--}11.00$ (7.50 ± 1.40) μm . Tectum perforate (Figs. 2, 3, 4) $0.15\text{--}0.40$ μm thick; outer columellae smaller than the supporting columellae (Figs. 2c, 3f, 4a, b) $0.15\text{--}0.90$ $\mu\text{m} \times 0.12\text{--}0.35$ μm , length/width = $1.30\text{--}3.70$; internal tectum more or less fragmented (Fig. 4a, b), $0.20\text{--}1.20$ μm thick; supporting columellae very variable in dimensions and shape, $1.10\text{--}4.00$ $\mu\text{m} \times 0.16\text{--}1.30$ μm , length/width = $1.00\text{--}21.40$, nearly straight (Figs. 1a, g, 2b, c, i) or more or less curved (Figs. 3e, f, 4a), and more or less ramified and branched (Figs. 2, 3, 4); foot layer $0.15\text{--}1.70$ μm thick; endexine generally thinner than the foot layer (Figs. 2b, i, 4a), $0.08\text{--}1.50$ μm thick. Spines conic with straight (Figs. 2c, 3a, d) or concave (Figs. 1b, 2d, e, g, 4b) sides, attenuate or contracted in an acute (Figs. 1b, 3d), acuminate (Fig. 3g), or obtuse (Fig. 2d) apex, spinular columellae longer than the supporting columellae (Fig. 4b), apex with a sub-apical cavity (Fig. 4b); length = $0.90\text{--}8.23$ (2.88 ± 0.90) μm , width at

the base = $1.90\text{--}11.54$ (4.79 ± 1.78) μm , length/width at the base = $0.29\text{--}1.38$ (0.61 ± 0.17) μm , number/100 μm^2 in the mesocolpium = $1\text{--}5$ (2.50 ± 0.90); spinular perforations = $0.10\text{--}1.14$ μm . Inter-spinular sculpture perforate (Figs. 2d, e, 3a, b) or microreticulate (Figs. 2f, g, 3c–f), sometimes rugulate-perforate (Fig. 2h), at least near the colpi margins; inter-spinular perforations = $0.09\text{--}0.70$ μm .

Pollen types

Dicoma anomala (incl. *Cloiselia carbonaria*, *C. olaeifolia*, *D. anomala*, *D. tomentosa*, “*Dicomopsis*” *welwitschii*, “*D.*” *baumii*, *Pasaccardoa jeffreyi*, *Macledium latifolium*, *M. sessiliflorum* and *M. spinosum*)

Pollen grains oblate-spheroidal to prolate, $P/E = 0.95\text{--}2.00$ (1.17 ± 0.10), subcircular to elliptical in mos, triangular-circular or circular in eos. $P = 34.00\text{--}75.00$ (51.70 ± 8.32) μm ; $E = 20.50\text{--}65.00$ (45.00 ± 7.90) μm . Mesoapertures lalongate (Fig. 1b, c, f), elliptic-shaped; endoapertures spindle-shaped, width of the endoapertures = $11.80\text{--}29.00$ (18.58 ± 4.16) μm . Supporting columellae relatively thick and straight, $1.30\text{--}2.90$ $\mu\text{m} \times 0.60\text{--}1.50$, length/width = $1.00\text{--}4.60$, densely distributed; exine thickness at the poles = $5.00\text{--}11.00$ (7.60 ± 1.30) μm . Spines with concave or straight sides, attenuated or rarely contracted in an acute apex, length = $0.90\text{--}3.70$ (2.34 ± 0.70) μm , width at the base = $2.00\text{--}7.00$ (4.42 ± 1.35) μm , length/width at the base = $0.29\text{--}0.90$ (0.54 ± 0.12), number of spines/100 $\mu\text{m}^2 = 1\text{--}5$ (2.34 ± 0.70), inter-spinular sculpture perforate, sometimes rugulate-perforate at least near the apertures.

Erythrocephalum longifolium (incl. *E. longifolium*, *E. marginatum* and *E. microcephalum*)

Pollen grains suboblate to prolate-spheroidal, $P/E = 0.87\text{--}1.07$ (0.97 ± 0.08), subcircular to elliptical in mos., triangular-circular or circular in eos. $P = 33.50\text{--}50.20$ (42.20 ± 3.79) μm ; $E = 36.00\text{--}51.70$ (43.50 ± 4.26) μm . Mesoapertures lolongate (Fig. 1d), elliptic-shaped; endoapertures narrowed in the centre and semi-circular in the ends (“butterfly-shaped”; Fig. 1d), width of the endoapertures = $5.90\text{--}20.40$ (11.90 ± 3.40) μm . Supporting columellae thin, loosely distributed, curved, $2.20\text{--}4.00$ $\mu\text{m} \times 0.15\text{--}0.20$ μm , length/width = $10\text{--}25$; exine thickness at the poles = $3.90\text{--}9.40$ (5.90 ± 1.10) μm . Spines with concave sides, attenuated in an acuminate apex, length = $2.70\text{--}8.20$ (5.07 ± 1.53) μm , width at the base = $3.10\text{--}11.50$ (7.00 ± 2.40) μm , length/width at the base = $0.55\text{--}1.37$ (0.75 ± 0.14), number of spines/100 $\mu\text{m}^2 = 1\text{--}3$ (1.73 ± 0.64), inter-spinular sculpture microreticulate.

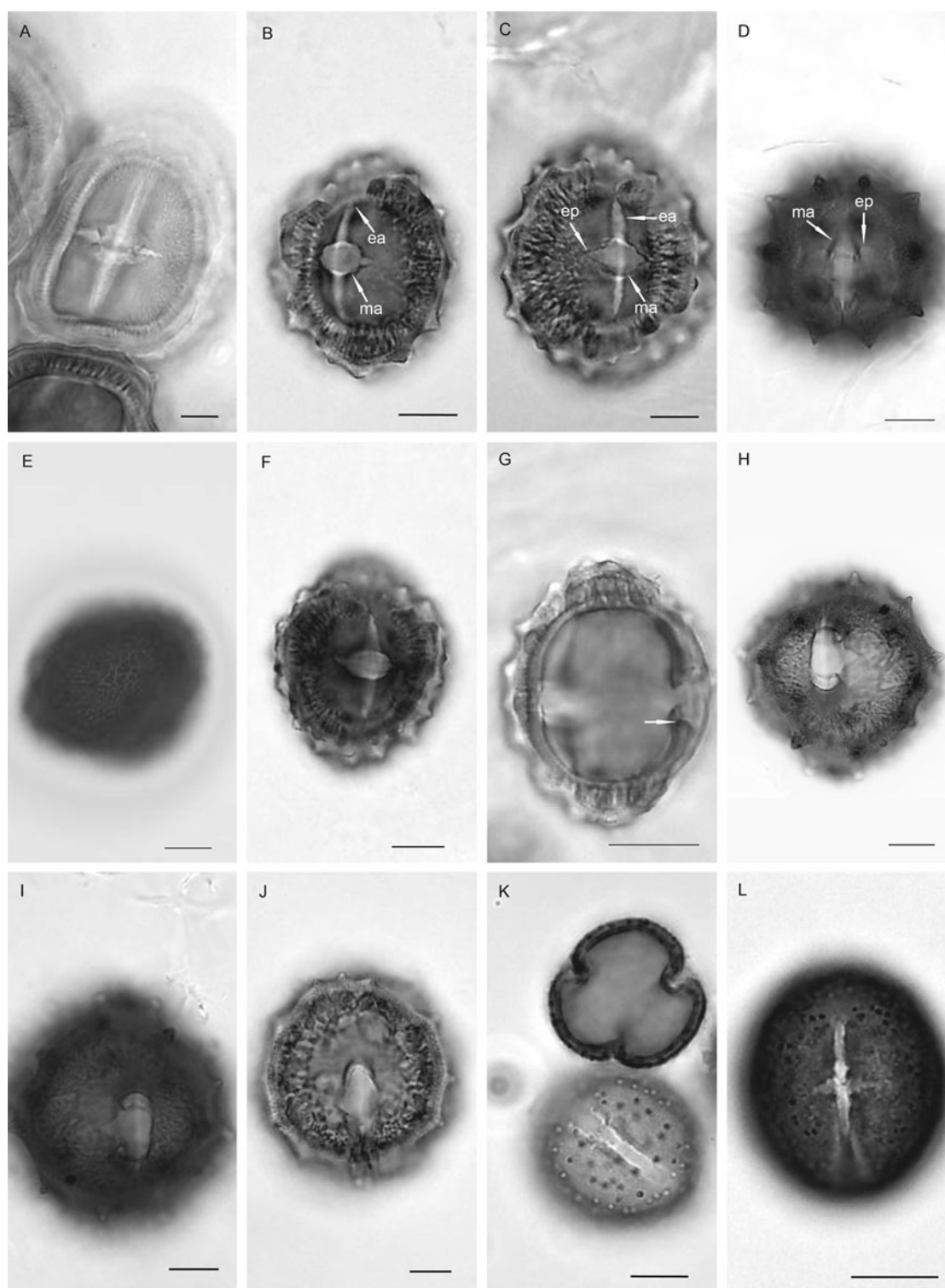


Fig. 1 LM micrographs. **a** *Cloiselia carbonaria*, equatorial superficial view, showing an aperture. **b** *Dicoma anomala*, equatorial superficial view, showing an aperture. **c** “*Dicomopsis*” *welwitschii*, equatorial superficial view, showing an aperture. **d** *Erythrocephalum longifolium*, equatorial superficial view, showing an aperture. **e** *Mactedium sessiliflorum*, equatorial superficial view of a mesocolpium. **f** *Pasaccardoa baumii*, equatorial superficial view of a mesocolpium. **g** *P. jeffreyi*, meridional optical section. **h** *Pleiotaxis*

rugosa, equatorial superficial view, showing an aperture. **i** *P. rugosa*, equatorial superficial view, showing an aperture. **j** *P. subpaniculata*, equatorial superficial view, showing an aperture. **k** *Brachylaena rotundata*, equatorial optical section and equatorial superficial view, showing an aperture, of two pollen grains. **l** *Tarchonanthus camphoratus* equatorial superficial view, showing an aperture. Arrow costae, *ea* ectoaperture, *ep* endoaperture, *ma* mesoaperture. **a–l** bars 10 µm

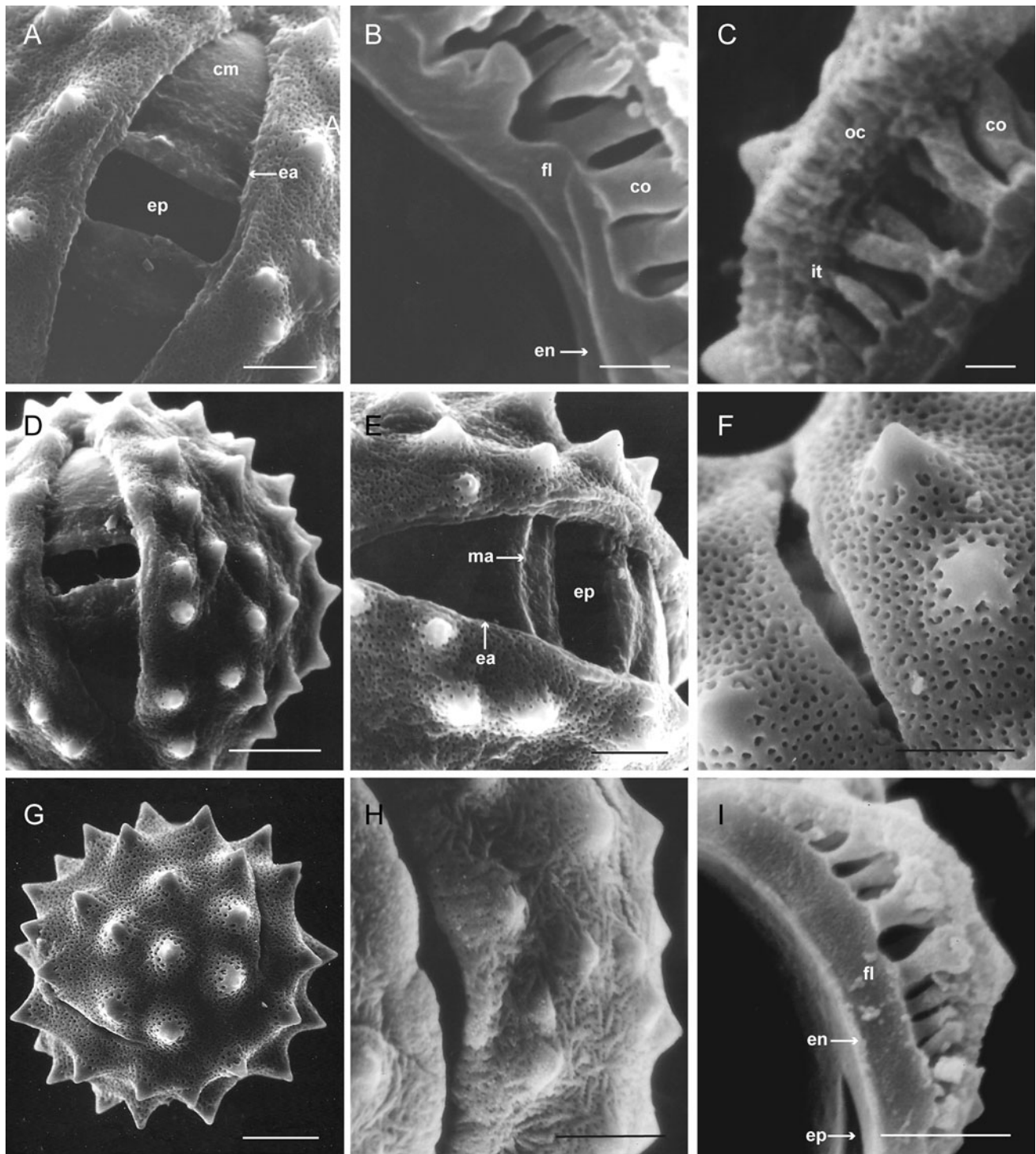


Fig. 2 SEM micrographs. *Cloiselia carbonaria*. **a** Equatorial view of an aperture. **b** Detail of a fractured exine. **c** *C. olaeifolia*, detail of a fractured exine. **d** *Dicoma anomala*, general equatorial view. **e** “*Dicomopsis*” *welwitschii*, equatorial view of an aperture. **f** *Erythrocephalum longifolium*, equatorial view of an aperture. **g** *E. marginatum*, general view. **h** *Macledium latifolium*, equatorial view of an

aperture. **i** *M. spinosum*, detail of a fractured exine. *cm* colpus membrane, *co* supporting columellae, *ea* ectoaperture, *en* endexine, *ep* endoaperture, *fl* foot layer, *it* internal tectum, *ma* mesoaperture, *oc* outer columellae. **a, e, f, h, i** bars 5 μ m; **b, c** bars 1 μ m; **d, g** bars 10 μ m

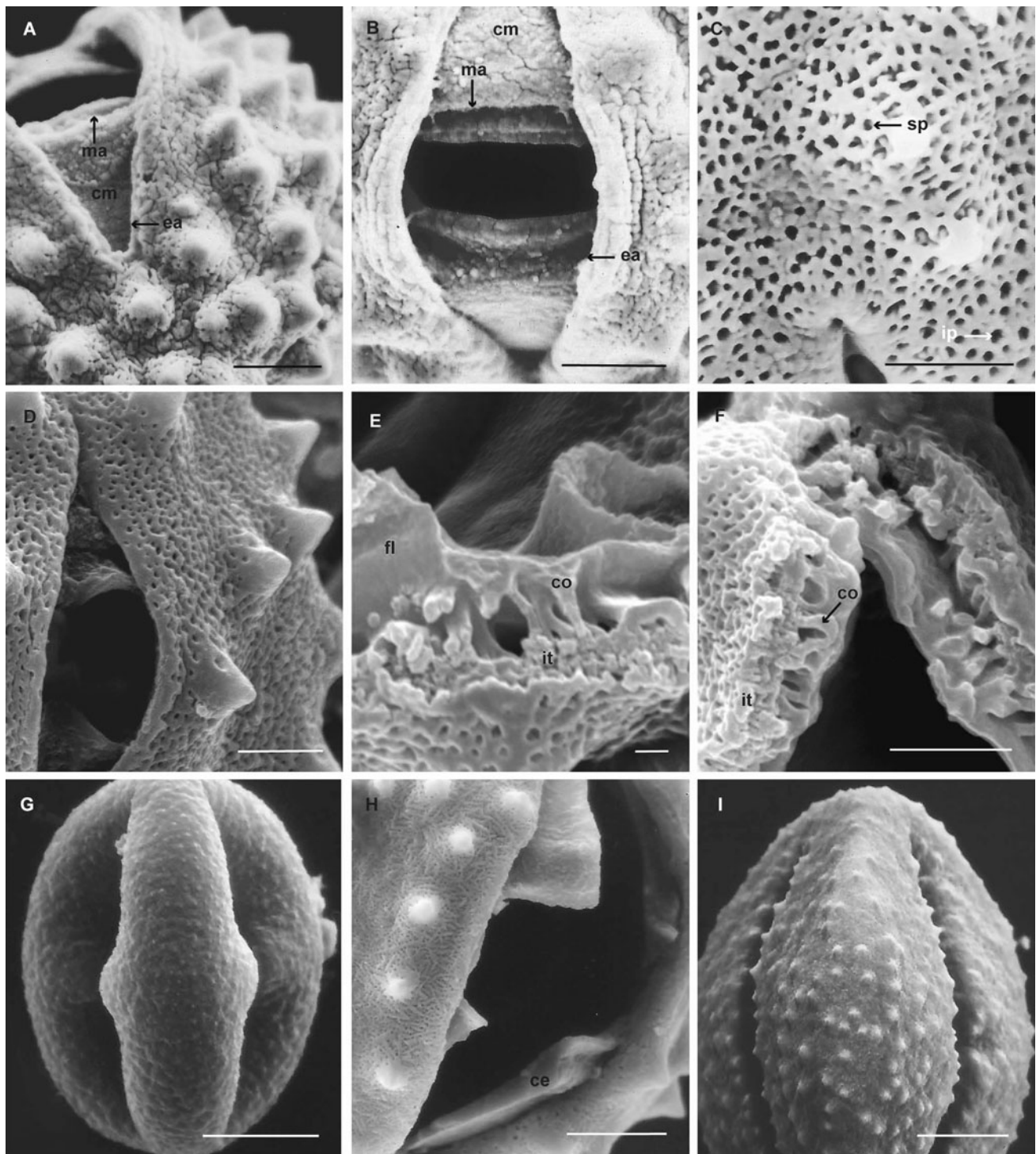


Fig. 3 SEM micrographs. *Pasaccardoa baumii*. **a** Polar view of the apocolpium and an aperture. **b** Equatorial view with an aperture. *Pleiotaxis rugosa*. **c** Polar view of the apocolpium and the apex of an aperture. **d** Equatorial view with an aperture. **e, f** *P. subpaniculata*, fractured exines. **g** *Chuquiraga jussieui*, equatorial view of a mesocolpius. **h** *Gongylolepis* sp. equatorial view with a fractured

aperture. **i** *Stenopadus* sp., equatorial view of a mesocolpius. *ce* costae, *cm* colpus membrane, *co* supporting columellae, *ea* ectoaperture, *ep* endoaperture, *fl* foot layer, *it* internal tectum, *ma* mesoaperture, *oc* outer columellae, *sp* spine perforation. **a–d, f** bars 5 μ m; **e** bar 1 μ m; **g–i** bars 10 μ m

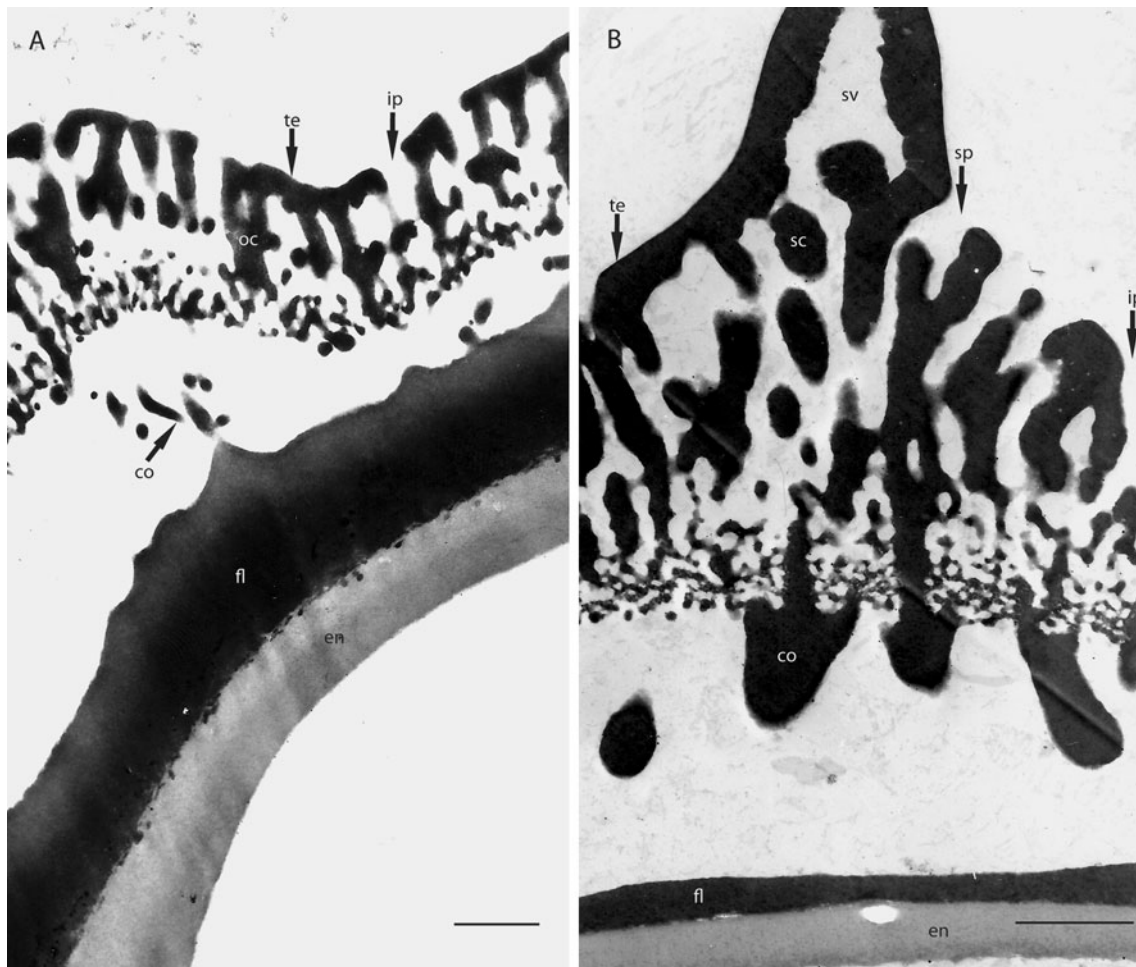


Fig. 4 TEM micrographs. Sections of the exine **a** *Erythrocephalum longifolium*. **b** *Pleiotaxis rugosa*. *co* supporting columellae, *en* endexine, *fl* foot layer, *oc* outer columellae, *ip* inter-spinular

perforations, *it* internal tectum, *sc* spinular columellae, *sp* spinular columellae, *sv* sub-apical cavity, *tc* tectal columellae, *te* tectum. **a, b** bars 1 µm

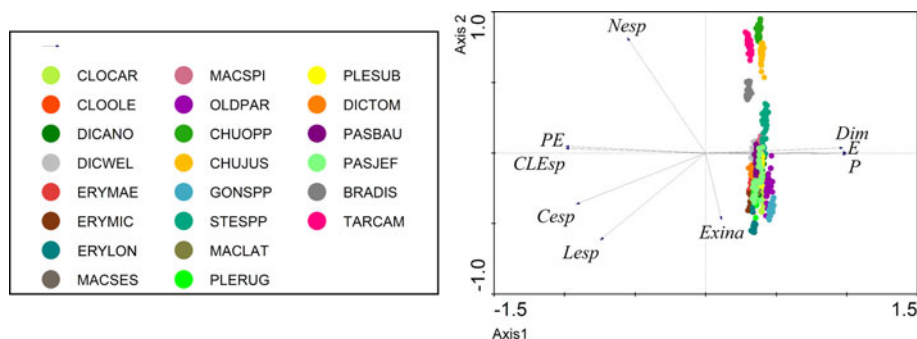


Fig. 5 PCA ordination diagram with the complete data-set showing specimen positions only. Traits and specimens are represented simultaneously in the PCA ordination diagram. In this diagram a few isolated groups were identified. BRADIS, *Brachylaena discolor*; CHUOPP, *Chquiraga oppositifolia*; CHUJUS, *C. jussieu*; CLOCAR, *Cloiselia carbonaria*; CLOOLE, *C. olaeifolia*; DICANO, *Dicoma anomala*; DICTOM, *D. tomentosa*; DICWEL, “*Dicomopsis*”

welwitschii; ERYLON, *E. longifolium*; ERYMAE, *E. marginatum*; ERYMIC, *E. microcephalum*; GONSPP, *Gongylolepis* sp.; MACLAT, *Macledium latifolium*; MACSES, *M. sessiliflorum*; MACSPI, *M. spinosum*; OLDPAR, *Oldenburgia paradoxa*; PASBAU, *Pascardoa baumii*; PASJEF, *P. jeffreyi*; PLERUG, *Pleiotaxis rugosa*; STESPP, *Stenopadus* sp.; TARCAM, *Tarchonanthus camphoratus*

Pleiotaxis rugosa (incl. *P. rugosa* and *P. subpaniculata*)

Pollen grains oblate-spheroidal to prolate-spheroidal, $P/E = 0.90\text{--}1.11$ (1.00 ± 0.04), subcircular or elliptical in mos, triangular-circular or circular in eos $P = 45.00\text{--}67.00$ (53.30 ± 6.24) μm ; $E = 44\text{--}68$ (53.00 ± 6.60) μm . Mesoapertures lolongate (Fig. 1i, j), elliptic-shaped; endoapertures spindle-shaped, width of the endoapertures = $12.00\text{--}22.00$ (16.20 ± 4.12) μm . Supporting columellae very variable in thickness and shape (Figs. 3h, i, 4b) some of them wide, more or less straight, ramified at the end, the others thin, loosely distributed and more or less curved, $1.10\text{--}2.10$ $\mu\text{m} \times 0.16\text{--}1.50$ μm , length/thickness = $1.50\text{--}10.00$; exine thickness at the poles = $6.20\text{--}11.00$ (8.30 ± 1.10) μm . Spines with concave sides, attenuated or contracted to an acute or obtuse apex, length = $2.00\text{--}5.70$ (3.40 ± 0.48) μm , width at the base = $1.90\text{--}7.50$ (4.40 ± 1.38) μm , length/width at the base = $0.46\text{--}1.32$ (0.80 ± 0.16), number of spines/100 μm^2 in the mesocolpium = $1\text{--}4$ (2.50 ± 0.83), inter-spinular sculpture microreticulate.

The following dichotomous key to Dicomeae pollen types is proposed:

1. Mesoapertures lalongate; supporting columellae thick, densely distributed and more or less straight; inter-spinular sculpture perforate or rugulate-perforate.....*Dicoma anomala* (subtribe Dicominae)
 - Mesoapertures lolongate; at least some of the supporting columellae thin, more or less loosely distributed and more or less curved; inter-spinular sculpture microreticulate (subtribe Pleiotaxinae)2
2. Endoapertures spindle shaped, spines obtuse or acute, contracted or attenuated at the apex.....*Pleiotaxis rugosa*
 - Endoapertures “butterfly” shaped, spines acuminate, attenuated at the apex.....*Erythrocephalum longifolium*

Discussion

The results we obtained for most of the pollen features (size, shape, apertures, sculpture) of the selected outgroups agree quite well with those reported by Parra and Marticorena (1972), Urtubey and Tellería (1998), Tellería (2008), and Ubierno et al. (2009). The same is true for the general exine patterns described by Skvarla et al. (1977). In

fact, the exines of *Erythrocephalum marginatum*, *E. microcephalum* (Ortiz and Pereira Coutinho 2001), *E. longifolium*, and *Pleiotaxis rugosa* have a typical anthemoid structural pattern (i.e. acaveate, with a thick foot layer, one layer of supporting columellae, several layers of outer columellae, and internal foramina absent). However, our results differ in some aspects from those reported by other authors.

For example, the dimensions we found for P and E and for the length of the spines are in general greater ($1.1\text{--}1.6$ times for P and E , $1.04\text{--}2.1$ for the length of the spines) than those reported by Hansen (1990) for the same species or genera. We believe, however, that these differences may arise because that author did not acetolyse the pollen grains he studied. We consider also the *Dicoma* general sculpture to be echinate and not areolate or verrucate (Perveen 1999).

The data published by the above mentioned authors, our own observations and PCA analysis (Fig. 5) revealed that the pollen grains of the studied species of tribes Oldenburgieae, Stifftieae, Tarchonantheae, and Wunderlichieae are all quite dissimilar from those of the Dicomeae. This is true (Table 3) of the values of P and E in *Gongylolepis* (Stifftieae) and *Oldenburgia* (Oldenburgieae) and of the number of spines in *Chuquiraga* (Barnadesieae), *Brachylaena*, and *Tarchonanthus* (Tarchonantheae). In fact, all are clearly larger than those of the Dicomeae (Table 1, Fig. 5). For these reasons, our results support the modern separation of the Dicomeae (Panero and Funk 2002) as an independent tribe.

The phylogenetic analysis of Panero and Funk (2008) using several cp DNA markers, and that of Ortiz et al. (2009) revealed that a clade comprising the Oldenburgieae and Tarchonantheae is the sister group of the Dicomeae. Our results give some support to this phylogenetic conclusion, because both tribes share a significative number of pollen characteristics with the Dicomeae (e.g. mesoapertures lolongate, similar to the Pleiotaxinae). It must be noted that the spine number and dimensions of the Oldenburgieae are clearly closer to the Dicomeae than those of the Tarchonantheae (Tables 1, 3, Fig. 5). Once again, these results agree with those cladograms, because the Oldenburgieae were placed in a closer position to the Dicomeae than the Tarchonantheae (Tables 2, 4, 5, 6).

Also, the palynological data undoubtedly support the division of the Dicomeae into two subtribes (Ortiz, unpublished). In fact, the mesoapertures are consistently lolongate in the Pleiotaxinae and lalongate in the Dicominae. In addition, at least some of the supporting columellae of the Pleiotaxinae are thinner, more curved and more loosely distributed (Figs. 3e, f, 4a, b) than those of the Dicominae (Figs. 1a, g, 2b, c, i). This is in agreement with micrographs of the exines of *Erythrocephalum marginatum* (SEM fractures and TEM) and *Dicoma zeyheri* (SEM

Table 1 Summary of pollen morphological quantitative characteristics for the Dicomaceae species examined and its monophyletic groups

Monophyletic group	Species	P (μm)	E (μm)	P/E	Exine thickness (μm)	Endoaperture width (μm)	N spines/100 μm ²	Spine length (μm)	Spine width (μm)	Spine length/width
<i>Cloiselia</i>	<i>C. carbonaria</i>	57.00–71.00 (64.53 ± 3.35)	50.00–65.00 (57.10 ± 3.36)	1.00–1.22 (1.13 ± 0.06)	6.80–10.20 (8.83 ± 0.91)	19.80–30.00 (25.20 ± 2.11)	1.00–3.00 (1.57 ± 0.57)	1.90–4.70 (3.20 ± 0.78)	3.60–6.90 (5.30 ± 0.94)	0.45–0.84 (0.59 ± 0.09)
	<i>C. oleaeifolia</i>	50.00–67.00 (58.77 ± 4.66)	45.00–60.00 (50.43 ± 4.13)	1.06–1.29 (1.17 ± 0.07)	5.00–7.10 (6.34 ± 0.61)	10.80–14.80 (12.66 ± 1.09)	2.00–5.00 (3.10 ± 0.71)	2.20–4.30 (3.10 ± 0.54)	4.10–7.30 (5.70 ± 0.90)	0.37–0.71 (0.55 ± 0.07)
	Total	50.00–71.00 (61.70 ± 5.00)	45.00–65.00 (53.77 ± 5.02)	1.00–1.29 (1.15 ± 0.07)	5.00–10.20 (7.58 ± 1.47)	10.80–30.00 (18.93 ± 6.54)	1.00–5.00 (2.33 ± 1.00)	1.90–4.70 (3.10 ± 0.70)	3.60–7.30 (5.50 ± 0.92)	0.37–0.84 (0.57 ± 0.09)
<i>Dicoma</i>	<i>D. anomala</i>	40.50–75.00 (52.10 ± 7.90)	38.50–65.00 (46.90 ± 5.38)	0.95–1.26 (1.11 ± 0.07)	6.20–11.00 (8.41 ± 1.14)	15.00–25.50 (19.39 ± 2.93)	1.00–4.00 (2.67 ± 0.76)	0.90–2.40 (1.64 ± 0.46)	2.20–6.00 (4.50 ± 0.90)	0.29–0.70 (0.48 ± 0.09)
	<i>D. tomentosa</i>	35.70–47.00 (42.00 ± 3.11)	20.50–36.50 (32.76 ± 3.16)	1.08–2.00 (1.29 ± 0.16)	5.00–7.50 (6.04 ± 0.63)	13.00–23.00 (17.36 ± 2.53)	2.00–4.00 (3.00 ± 0.37)	2.60–3.00 (2.84 ± 0.11)	4.50–7.00 (6.28 ± 0.66)	0.39–0.62 (0.46 ± 0.06)
	Total	35.70–75.00 (47.04 ± 7.80)	20.50–65.00 (39.83 ± 8.36)	0.95–2.00 (1.20–0.15)	5.00–11.00 (7.23 ± 1.50)	13.00–25.50 (18.44 ± 2.81)	1.00–4.00 (2.83 ± 0.62)	0.90–3.00 (2.24 ± 0.69)	2.20–7.00 (4.88 ± 1.61)	0.29–0.70 (0.47 ± 0.08)
“ <i>Dicomopsis</i> ”	<i>Dicoma welwitschii</i>	36.10–47.00 (50.00 ± 2.80)	32.50–39.00 (35.2 ± 1.47)	1.03–1.32 (1.17 ± 0.08)	6.50–11.00 (7.95 ± 1.02)	12.00–20.00 (15.93 ± 1.80)	2.00–5.00 (3.07 ± 0.78)	1.20–3.00 (1.98 ± 0.46)	2.50–5.10 (3.71 ± 0.63)	0.33–0.75 (0.54 ± 0.13)
	<i>Pascardoa baumii</i>	43.50–59.70 (49.62 ± 3.65)	36.50–48.50 (42.30 ± 2.61)	1.05–1.32 (1.17 ± 0.06)	5.50–9.00 (7.24 ± 0.84)	11.80–21.00 (18.15 ± 2.52)	2.00–4.00 (3.20 ± 0.66)	1.60–3.10 (2.28 ± 0.44)	2.70–5.50 (3.94 ± 0.72)	0.36–0.83 (0.59 ± 0.11)
	Total	36.10 ± 59.70 (45.30 ± 5.42)	32.50–48.50 (38.72 ± 4.15)	1.03–1.32 (1.17 ± 0.07)	5.50–11.00 (7.84 ± 1.44)	12.70–27.00 (20.55 ± 4.15)	1.00–4.00 (1.90 ± 0.88)	1.10–2.90 (2.16 ± 0.47)	2.10–6.50 (4.31 ± 1.16)	0.39–0.90 (0.52 ± 0.11)
<i>Pascardoa</i>	<i>P. jeffreyi</i>	53.00–65.00 (56.97 ± 3.20)	46.00–56.00 (50.58 ± 2.70)	1.00–1.21 (1.13 ± 0.05)	6.00–9.50 (7.58 ± 0.94)	14.50–29.00 (21.84 ± 3.78)	2.00–3.00 (2.83 ± 0.38)	1.60–3.70 (2.52 ± 0.53)	3.50–7.00 (5.03 ± 0.93)	0.32–0.71 (0.51 ± 0.09)
	<i>M. latifolium</i>	49.50–60.00 (54.27 ± 2.72)	37.00–48.00 (43.29 ± 2.31)	1.21–1.46 (1.25 ± 0.05)	7.00–10.40 (8.60 ± 0.88)	13.00–20.50 (17.00 ± 1.91)	1.00–3.00 (1.97 ± 0.67)	1.30–2.10 (2.09 ± 0.37)	2.10–3.80 (2.80 ± 0.37)	0.57–0.90 (0.74 ± 0.09)
	Total	43.50–65.00 (53.65 ± 4.34)	37.00–56.00 (46.40 ± 3.95)	0.98–1.46 (1.16 ± 0.09)	6.00–10.40 (7.86 ± 1.04)	12.00–21.00 (17.61 ± 2.11)	2.00–5.00 (3.23 ± 0.82)	1.10–2.50 (1.64 ± 0.32)	2.00–5.70 (3.65 ± 1.13)	0.30–0.67 (0.48 ± 0.12)
<i>Erythrocephalum</i> + <i>Pleiotaxis</i>	<i>E. longifolium</i>	43.10–50.20 (46.69 ± 2.22)	38.50–49.00 (45.31 ± 2.41)	0.98–1.27 (1.10 ± 0.06)	6.00–9.00 (7.40 ± 0.88)	12.00–21.00 (17.61 ± 2.11)	2.00–5.00 (3.23 ± 0.82)	1.10–2.50 (1.64 ± 0.32)	2.00–5.70 (3.65 ± 1.13)	0.30–0.67 (0.48 ± 0.12)
	<i>E. marginatum</i>	33.70–46.30 (40.93 ± 3.03)	36.90–46.30 (41.64 ± 2.74)	0.91–1.03 (0.98 ± 0.03)	5.10–8.60 (6.10 ± 0.89)	5.90–15.00 (9.49 ± 2.09)	1.00–3.00 (1.69 ± 0.54)	2.70–4.70 (3.64 ± 0.53)	3.10–7.50 (4.84 ± 1.17)	0.55–1.37 (0.78 ± 1.18)
	<i>E. microcephalum</i>	34.00–45.10 (40.53 ± 3.22)	36.00–46.70 (41.30 ± 2.88)	0.87–1.07 (0.98 ± 0.05)	3.90–9.41 (5.15 ± 1.29)	9.40–17.30 (12.81 ± 2.42)	2.00–3.00 (2.33 ± 0.49)	5.14–6.86 (6.15 ± 0.51)	8.00–10.29 (8.87 ± 0.70)	0.60–0.82 (0.70 ± 0.07)
<i>Erythrocephalum</i>	Total	33.70–50.20 (42.24 ± 3.80)	36.00–51.70 (43.47 ± 4.30)	0.87–1.07 (0.97 ± 0.04)	3.90–9.41 (5.95 ± 1.12)	5.90–20.38 (11.88 ± 3.38)	1.00–3.00 (1.73 ± 0.64)	2.74–8.23 (5.07 ± 1.53)	3.10–11.54 (7.02 ± 2.39)	0.55–1.37 (0.75 ± 0.14)
	<i>P. rugosa</i>	47.00–54.00 (49.14 ± 1.65)	46.00–55.00 (49.51 ± 2.07)	0.94–1.04 (0.99 ± 0.02)	6.50–11.00 (8.05 ± 1.09)	12.00–17.50 (14.58 ± 1.43)	1.00–3.00 (2.17 ± 0.70)	2.00–5.70 (4.18 ± 0.74)	2.50–7.50 (5.11 ± 1.23)	0.48–1.00 (0.84 ± 0.12)
	<i>P. subpaniculata</i>	45.00–67.00 (57.50 ± 6.35)	43.50–68.00 (53.36 ± 7.18)	0.90–1.11 (1.01 ± 0.05)	6.20–10.50 (8.60 ± 1.03)	12.00–22.00 (17.74 ± 2.70)	1.00–4.00 (2.87 ± 0.82)	2.00–4.40 (2.66 ± 0.44)	1.90–6.80 (3.73 ± 1.18)	0.46–1.32 (0.76 ± 0.18)
<i>Pleiotaxis</i>	Total	45.00–67.00 (53.32 ± 6.24)	43.50–68.00 (53.43 ± 6.47)	0.90–1.11 (1.00 ± 0.04)	6.20–11.00 (8.33 ± 1.09)	12.00–22.00 (16.16 ± 4.12)	1.00–4.00 (2.52 ± 0.83)	2.00–5.70 (3.42 ± 0.98)	1.90–7.50 (4.42 ± 1.38)	0.46–1.32 (0.80 ± 0.16)
	Total	33.70–67.00 (47.83 ± 7.58)	36.00–68.00 (48.51 ± 7.48)	0.87–1.11 (0.99 ± 0.04)	3.90–11.00 (7.10 ± 1.60)	5.90–22.00 (14.04 ± 3.72)	1.00–4.00 (2.00 ± 0.84)	2.00–8.23 (4.24 ± 1.52)	1.90–11.54 (5.71 ± 2.74)	0.46–1.37 (0.77 ± 0.15)

Table 2 Summary of pollen morphological qualitative characteristics for the Dicomeae species examined and its monophyletic groups

Monophyletic group	Species	Spine apex	Spine transition base/apex	Inter-spinular sculpture	Mesoaperture shape	Endoaperture shape
<i>Cloiselia</i>	<i>C. carbonaria</i>	Obtuse (ca. 1/2) or acute (ca. 1/2)	Contracted	Perforate	Lalongate, elliptic	Lalongate spindle-shaped
	<i>C. oleaeifolia</i>	Acute	Contracted	Perforate	Lalongate, elliptic	Lalongate, spindle-shaped
	Total	Mainly acute (ca. 3/4), some obtuse (ca. 1/4)	Contracted	Perforate	Lalongate, elliptic	Lalongate, spindle-shaped
<i>Dicoma</i>	<i>D. anomala</i>	Acute	Attenuate	Perforate	Lalongate, elliptic	Lalongate, spindle-shaped
	<i>D. tomentosa</i>	Acute	Attenuate	Perforate	Lalongate, elliptic	Lalongate, spindle-shaped
	Total	Acute	Attenuate	Perforate	Lalongate, elliptic	Lalongate, spindle-shaped
“ <i>Dicomapsis</i> ”	<i>Dicoma welwitschii</i>	Acute	Attenuate	Perforate	Lalongate, elliptic	Lalongate, spindle-shaped
	<i>Pasaccardoa baumii</i>	Acute	Attenuate	Perforate or rugulate-perforate	Lalongate, elliptic	Lalongate, spindle-shaped
	Total	Acute	Attenuate	Perforate or rugulate-perforate	Lalongate, elliptic	Lalongate, spindle-shaped
<i>Pasaccardoa</i>	<i>P. jeffreyi</i>	Acute	Attenuate	Perforate	Lalongate, elliptic	Lalongate, spindle-shaped
	<i>M. latifolium</i>	Acute	Contracted	Perforate or rugulate-perforate	Lalongate, elliptic	Lalongate, spindle-shaped
	<i>M. sessiliflorum</i>	Acute	Attenuate	Perforate	Lalongate, elliptic	Lalongate, spindle-shaped
<i>Macledium</i>	<i>M. spinosum</i>	Acute	Attenuate	Perforate	Lalongate, elliptic	Lalongate, spindle-shaped
	Total	Acute	Attenuate or contracted	Perforate or rugulate-perforate	Lalongate, elliptic	Lalongate, spindle-shaped
<i>Erythrocephalum</i> + <i>Pleiotaxis</i>	<i>E. longifolium</i>	Acuminate	Attenuate	Microreticulate	Lalongate, elliptic	Lalongate, “butterfly”-shaped
	<i>E. marginatum</i>	Acuminate	Attenuate	Microreticulate	Lalongate, elliptic	Lalongate, “butterfly”-shaped
	<i>E. microcephalum</i>	Acuminate	Attenuate	Microreticulate	Lalongate, elliptic	Lalongate, “butterfly”-shaped
<i>Erythrocephalum</i>	Total <i>Erythrocephalum</i>	Acuminate	Attenuate	Microreticulate	Lalongate, elliptic	Lalongate, “butterfly”-shaped
	<i>P. rugosa</i>	Obtuse or acute	Contracted	Microreticulate	Lalongate, elliptic	Lalongate, spindle-shaped
	<i>P. subpaniculata</i>	Obtuse or acute	Contracted or attenuate	Microreticulate	Lalongate, elliptic	Lalongate, spindle-shaped
<i>Pleiotaxis</i>	Total <i>Pleiotaxis</i>	Obtuse	Contracted or attenuate	Microreticulate	Lalongate, elliptic	Lalongate, spindle-shaped
	Total	Acuminate or obtuse	Attenuate or contracted	Microreticulate	Lalongate, elliptic	Lalongate, “butterfly”-shaped or spindle-shaped

Table 3 Pollen quantitative characteristics of some tribes related to the Dicomeae

Tribe	Taxa	P (μm)	E (μm)	P/E	Exine thickness (μm)	Endoaperture width (μm)	N spines/100 μm ²	Spine length (μm)	Spine width (μm)	Spine length/spine width
Barnadesieae	<i>Chuquiraga jussieui</i>	47.00–58.50 (53.00 ± 2.65)	41.20–49.00 (45.00 ± 1.60)	1.08–1.36 (1.19 ± 0.07)	4.20–6.90 (5.40 ± 0.70)	20.00–30.00 (24.00 ± 2.90)	10.00–22.00 (17.30 ± 2.80)	0.22–0.44 (0.33 ± 0.08)	0.89–1.11 (1.00 ± 0.08)	0.25–0.40 (0.32 ± 0.06)
	<i>Chuquiraga oppositifolia</i>	34.00–52.00 (44.50 ± 3.83)	33.00–46.00 (39.00 ± 2.40)	0.99–1.38 (1.15 ± 0.09)	4.70–7.00 (5.60 ± 0.50)	15.00–25.00 (19.80 ± 2.79)	18.00–30.00 (24.20 ± 2.89)	0.11–0.23 (0.17 ± 0.05)	0.34–0.45 (0.40 ± 0.05)	0.32–0.51 (0.42 ± 0.08)
	Total	34.00–58.50 (48.70 ± 5.39)	33.00–49.00 (44.50 ± 1.55)	0.99–1.38 (1.17 ± 0.08)	4.20–7.00 (5.50 ± 0.60)	15.00–30.00 (21.53 ± 3.74)	10.00–30.00 (20.70 ± 4.49)	0.11–0.44 (0.25 ± 0.10)	0.34–1.11 (0.70 ± 0.31)	0.25–0.51 (0.37 ± 0.08)
	<i>Oldenburgia paradoxa</i>	79.00–127.00 (101.00 ± 10.70)	70.00–107.00 (87.00 ± 9.70)	1.06–1.32 (1.16 ± 0.07)	7.50–13.00 (9.60 ± 1.40)	22.00–33.00 (28.00 ± 3.60)	1.00–3.00 (1.83 ± 0.53)	2.00–4.70 (3.00 ± 0.60)	2.80–7.00 (5.50 ± 1.30)	0.36–1.00 (0.56 ± 0.13)
Stiffiteae	<i>Gongylolepis</i> sp.	103.00–131.00 (113.00 ± 7.12)	90.00–113.00 (100.00 ± 7.10)	1.05–1.22 (1.14 ± 0.04)	7.20–11.00 (9.50 ± 1.00)	27.00–41.00 (35.00 ± 4.40)	1.00–1.00 (1.00 ± 0.00)	2.10–5.30 (3.68 ± 0.56)	4.80–8.20 (6.39 ± 1.03)	0.39–0.75 (0.59 ± 0.09)
	<i>Brachylaena discolor</i>	29.00–35.00 (32.20 ± 1.35)	28.00–33.00 (30.00 ± 1.40)	0.92–1.23 (1.06 ± 0.06)	1.50–3.00 (2.00 ± 0.30)	6.50–11.00 (8.29 ± 1.29)	6.00–10.00 (7.67 ± 1.12)	0.80–1.60 (1.16 ± 0.24)	0.70–1.20 (0.88 ± 0.15)	0.91–1.88 (1.34 ± 0.28)
Tarchonantheae	<i>Tarchonanthus camphoratus</i>	28.00–39.00 (33.00 ± 2.84)	25.00–33.00 (29.00 ± 2.10)	1.04–1.28 (1.14 ± 0.06)	2.50–3.50 (2.40 ± 0.50)	8.50–15.00 (10.76 ± 1.76)	13.00–26.00 (20.40 ± 2.82)	0.50–0.80 (0.66 ± 0.09)	0.40–1.00 (0.65 ± 0.18)	0.70–2.00 (1.08 ± 0.33)
	Total	28.00–39.00 (32.60 ± 2.25)	25.00–33.00 (30.00 ± 1.09)	0.92–1.28 (1.10 ± 0.07)	1.50–3.50 (2.40 ± 0.50)	6.50–15.00 (9.53 ± 1.97)	6.00–26.00 (14.00 ± 6.75)	0.50–1.60 (0.66 ± 0.09)	0.40–1.20 (0.76 ± 0.20)	0.70–2.00 (1.21 ± 0.33)
Wunderlichieae	<i>Stenopadus</i> sp.	72.00–84.00 (78.00 ± 3.35)	50.00–67.00 (58.00 ± 4.50)	1.07–1.54 (1.34 ± 0.10)	5.40–8.50 (6.80 ± 0.80)	14.00–33.00 (22.00 ± 6.30)	4.00–7.00 (5.23 ± 0.97)	0.70–1.70 (1.04 ± 0.29)	1.40–5.70 (2.52 ± 1.42)	0.24–0.60 (0.47 ± 0.12)

fractures) published by Blackmore et al.'s (2010) and with Stix's (1960) LM observations, this last author having pointed out the presence of supporting columellae of the thick, straight, and dense type in *Dicoma* and a thin, curved and spaced type in *Erythrocephalum*. In addition, the inter-spinular sculpture of the Pleiotaxinae is always microreticulate, and the inter-spinular sculpture of the Dicominae is predominantly perforate (these two kinds of ornamentation also agree with the SEM micrographs of Blackmore et al. 2010) or rarely rugulate-perforate. Moreover, the average and extreme values for the former subtribe of spine length and width and their ratio are, respectively, greater and smaller than those of the Dicominae (Table 1). The existence of two distinct mesoaperture shapes, sub-patterns of the anthemoid structure and patterns of sculpture in the Dicomeae could give some support to a hypothetical elevation of the Pleiotaxinae to the tribal rank (Ortiz et al. 2009). It is noteworthy that, although the qualitative data were not included, the PCA also revealed a trend for the separation of Dicominae and Pleiotaxinae (Fig. 6).

Although the existence of the Pleiotaxinae is well supported by the palynological data, it must be noted that the endoaperture and most of the spine shapes of the pollen grains of *Pleiotaxis* and *Erythrocephalum* are quite distinct, and the quantitative values of most of their characters differ substantially (Table 1). For those reasons we propose two distinct pollen types for the Pleiotaxinae we studied.

Interestingly, within the Dicominae, some of the *Cloiselia* palynological features are also somewhat distinct from those of all the other taxa we studied, and for several characters they represent an intermediate condition between the other Dicominae and the Pleiotaxinae. In fact, the *Cloiselia* pollen grains have maximum values of *P* and *E* larger than those of the other Dicominae (Table 1). In addition, the average spine dimensions (length and width at the base) for this genus are closer to the spine dimensions of the Pleiotaxinae (Table 1). Nevertheless, the existence of a continuous transition among the quantitative pollen characters of the Dicominae species we examined (Table 1) did not enable us to define two pollen types for this subtribe. On the contrary, we consider that our results support the existence of a large clade comprising the *Macleodium*, *Pasaccardoa*, "*Dicomopsis*", *Dicoma*, and *Cloiselia* clades (Ortiz 2000; Ortiz et al. 2009), because the pollen grains of all these genera share important features, namely thick, more or less straight, dense supporting columellae, lalongate mesoapertures, and perforate (rarely rugulate-perforate) inter-spinular sculpture.

The average values we found for the equatorial diameter (46.00 μm), the exine thickness (7.50 μm), and the ratio "*E*/exine thickness" (6.13) of the Dicomeae are quite close to those indicated by Bolick (1991) for both the Mutisieae

Table 4 Summary of pollen morphological qualitative characteristics for the Barnadesieae, Oldenburgieae, Stiffieae, Tarchonantheae, and Wunderlichieae species examined

Tribe	Taxa	Spine apex	Spine transition base/apex	Inter-spinular sculpture	Mesoaperture shape	Endoaperture shape
Barnadesieae	<i>Chuquiraga jussieui</i>	Acute	Attenuate	Psilate	Non-observable	Lalongate, spindle-shaped
	<i>Chuquiraga oppositifolia</i>	Acute	Attenuate	Psilate	Non-observable	Lalongate, spindle-shaped
	Total	Acute	Attenuate	Psilate	Non-observable	Lalongate, spindle-shaped
Oldenburgieae	<i>Oldenburgia paradoxa</i>	Acute	Attenuate	Scabrate	Lolongate, elliptic	Lalongate, spindle-shaped
Stiffieae	<i>Gongylolepis</i> sp.	Acute	Attenuate	Rugulate	Lalongate, elliptic	Lalongate, spindle-shaped
Tarchonantheae	<i>Brachylaena discolor</i>	Acute	Attenuate	Scabrate	Lolongate, elliptic	Lalongate, spindle-shaped
	<i>Tarchonanthus camphoratus</i>	Acute (ca. 4/5) or obtuse (ca. 1/5)	Attenuate	Scabrate	Non-observable	Lalongate, spindle-shaped
	Total	Mainly acute, some obtuse	Attenuate	Scabrate	Lolongate, elliptic, or non-observable	Lalongate, spindle-shaped
Wunderlichieae	<i>Stenopadus</i> sp.	Acute	Attenuate	Scabrate	Non-observable	Lalongate, spindle-shaped

Table 5 Eigenvalues and percentage of variance explained by the first three factors in principal-components analysis of the pollen data of the taxa of Dicomeae

Axis	Eigenvalue	% Variance	Cumulative % variance
1	0.780	78.20	78.20
2	0.098	9.80	88.00
3	0.062	6.20	94.20

Table 6 Eigenvalues and percentage of variance explained by the first three factors in principal-components analysis of the pollen data of the taxa of Dicomeae, Barnadesieae, Oldenburgieae, Stiffieae, and Tarchonantheae

Axis	Eigenvalue	% Variance	Cumulative % variance
1	0.651	65.10	65.10
2	0.241	24.10	89.20
3	0.050	5.00	94.20

(respectively: 43.10, 6.50, and 6.60 μm) and Cardueae (respectively: 49.10, 6.50, and 7.50 μm). Taking into account that the Mutisieae sensu lato included the Dicomeae, and that the Cardueae belong to a clade that includes also the Dicomeae (Funk et al. 2009; Ortiz et al. 2009), these similarities seem to be quite logical. Our results support that author's conclusion that pollen diameter and exine thickness in the Asteraceae are correlated with the exine ultrastructure type (anthemoid, in this case).

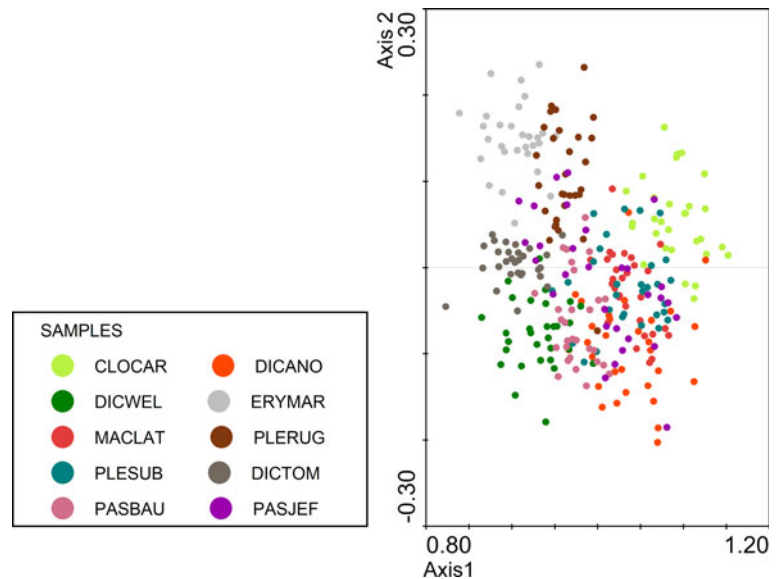
Some considerations about the palynocology of the Dicomeae must be made. The existence in its exine of a

tensile system of both outer and supporting columellae constitutes good protection against compressive forces (Bolick 1978, 1991). It must be pointed out that the thin supporting columellae of some Dicomeae (Pleiotaxinae, mainly *Erythrocephalum*) certainly enhance the efficiency of the harmomegathic mechanisms by increasing the flexibility of the exine (Muller 1979). Also, the presence of a multi-stratified (fragmented) internal tecta and the absence of internal foramina are effective defences against the loss of water by the male gametophyte, a clear ecological advantage in the xeric habitats where most of the species of Dicomeae live.

The main conclusions of this work can be summarized as follows:

1. All the taxa of Dicomeae share the same general pattern of structure (anthemoid);
2. the Dicominae and Pleiotaxinae can be easily separated by their pollen morphology;
3. the hypothetical elevation of Pleiotaxinae to tribe status is supported by this study;
4. the pollen morphology of the taxa of Dicominae is quite similar, although *Cloiselia* is somewhat closer to the Pleiotaxinae than the other Dicominae;
5. within the Pleiotaxinae, the pollen grains of *Erythrocephalum* and *Pleiotaxis* share many important similarities, but also some significant differences;
6. within the tribes closely related to Dicomeae, the pollen morphology of the Oldenburgieae and Tarchonantheae (in this order) is closest to that of that tribe;
7. the pollen morphology of the related tribes Stiffieae, Oldenburgieae, Tarchonantheae, and Wunderlichieae is quite different from that of the Dicomeae.

Fig. 6 Additional PCA was performed, removing isolated groups to identify others. Results from the PCA performed over the data-set restricted to Dicomeae is shown. CLOCAR, *Cloiselia carbonaria*; DICANO, *Dicoma anomala*; DICTOM, *D. tomentosa*; DICWEL, “*Dicomopsis*” *welwitschii*; ERYMAR, *Erythrocephalum marginatum*; MACLAT, *Macledium latifolium*; PASBAU, *Pasaccardoa baumii*; PASJEF, *P. jeffreyi*; PLERUG, *Pleiotaxis rugosa*; PLESUB, *Pleiotaxis subpaniculata*



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Appendix: Studied specimens

Brachylaena discolor DC.

Moçambique. Ilha de Inhaca, 3.IX.1975, A. Reis Moura 695 (COI). Ilha de Inhaca, 27.VII.1982, D. Zuzunga 357 (COI).

Chuquiraga jussieui J. F. Gmel.

Bolivia. LaPaz, Murillo, entre LaPaz y Palca, 25. II. 1979, A. Ceballos, Charpin, F. Casas & V. Bermejo BO-522 (MA).

Chuquiraga oppositifolia D. Don

Chile. Prov. Colchaqua, San Fernando, Termas del Flaco, 13. XI. 1983, G. Montero 12450 (MA).

Cloiselia carbonaria S. Moore

Madagascar. Vallées du Mangoky et de L' Isahaina, 10/1933, 11294 (K). La Table, Tuléar, 11. XI. 1962, F. Chauvet 359 (K). Plateau calcaire Mahafaly entre Stampé et Ampanily, 16. X. 1970, M. Keraudren-Aymonin & G. Aymonin 24572 (K).

Cloiselia oleifolia (Humbert) S. Ortiz

Madagascar. Collines des environs de Berlaner, V. 1902, H. Bâthie 1428 (K). Environs de Tuléar, 6. XI. 1960, J. Leandri, Ratoto, Jean de Dieu 3730 (K).

Dicoma anomala Sond.

Zaire. Haut-Shaba, Plateau de la Monica. **Angola.** Huíla, Humpata, 9. VI. 1960, Teixeira & Andrade 4740

(COI). Huambo, Nova Lisboa, Chianga, 6. VII. 1970, Silva 3181 (COI).

Dicoma tomentosa Cass.

Angola. Mossâmedes, 26. IV. 1938, Abreu 81 (COI).

Mozambique. Tete, pr. Missão de Boroma, 21. VI. 1941, A. R. Torre 2921 (COI).

“*Dicomopsis*” *welwitschii* (= *Dicoma welwitschii* O. Hoffm.)

Angola. Benguela, Cacomba, 26. VII. 1905, Gossweiler 1798 (COI). Iter. Angolense, s. d., Welwitsch 3629 (COI). Quanza Sul, 24. VI. 1942, Excell & Mendonça 3069 (COI). Huambo, Nova Lisboa, 15. VI. 1971, M. da Silva 3668 (COI).

Erythrocephalum longifolium Benth. ex Oliv.

Kenia. 8. I. 1969, Mouarig 114 (K). N'Gomeni, Tanga, 18. IV. 1964, H. Faackman, 3980 (K). **Malawi.** Musidi Road, 8. I. 1969, Mouarig 114 (K).

Erythrocephalum marginatum (O. Hoffm.) S. Ortiz & A. P. Cout.

Tanzania. Dakara Ranch, 26. IV. 1988, Pócs 86 (K).

Erythrocephalum microcephalum Dandy

Kenya. 35 miles SW of Kinui, 30. I. 1957, Bogdon 63 (K). **Tanganyica.** Kikori, 20. II. 1930, Burt 64 (K).

Uganda. I. 1922, Dummer 5015 (K).

Gongyolepis sp.

Venezuela. Territorio Federal del Amazonas, Depto. Atabapo, Cerro Duida, Rio Negro, 19. XI. 1991, J. Fuertes & J. M. Cardiel 1267 (MA 524239)

Macledium latifolium (DC.) S. Ortiz

South Africa. Potberg, Bredasdorp, s. d., Acocks 23014 (PRE).

Macledium sessiliflorum (Harv.) S. Ortiz

Tanzania. Kilosa District, near Ruaha River, 12 km W. of Kidatu Bridge, 4. IX. 1970, M. Thulin & B. Mhoro 856

- (K). 26 km SE of Mtwara, 6 km SW of Msimbati, 1. XI. 1977, R. Wingfield 4416 (K). Magubie, Mamboya, VIII. 1930, A. Haarer 1835 (K).
Macledium spinosum (L.) S. Ortiz
South Africa. Noukloof Nature Reserve, 13. XI. 1982, D. F. Laidler 432 (PRE).
Oldenburgia paradoxa Less.
South Africa. Montagu, II.1983, Kotze & Parker 341 (NBG). Western Cape Province, Oudtshoorn district, Cra-dockberg, George, IV. 1963, Esterhuysen 30160 (S).
Pasaccardoa baumii O. Hoffm.
Angola. Benguela, 1910, Gossweiler 3017 (K). Bié, Cuamba, 18. X. 1966, R. Monteiro & Murta 2024 (COI). Bié, Chitembo, Anhara do Rio Cutato, a 7 km de Chuinda, 19. X. 1966, J. B. Teixeira et al. 10780 (COI).
Pasaccardoa jeffreyi Wild
Angola. Lunda, Xassengue, IV. 1937, H. W. (COI). Lunda, Xassengue, IV. 1937, Gossweiler 11799 (COI).
Pleiotaxis rugosa O. Hoffm.
Angola. Lunda, Dala, 18. VIII. 1927, Carriço & Mendonça 362 (COI). Huambo, 17. V. 1969, Moreno s. n. (COI). Huambo, pr. Rio Cuando, 17. V. 1969, Moreno, s. n. (COI).
Pleiotaxis subpaniculata Chiov.
Angola. Nova Lisboa, Sacaala, 18. II. 1962, Murta 103 (COI).
Stenopadus sp.
Venezuela. Territorio Federal del Amazonas, Depto. Atabapo, P. N. Duida-Marahuaca, Cerro Duida, Rio Negro, 19. XI. 1991, J. Pedrol 4585 JP (MA 524700).
Tarchonanthus camphoratus L.
Zimbabwe. Southern Zimbabwe, western slope of Dokodoko Mountain, 1 mile from Hot Springs, 23 X. 1948, N. C. Chase 921 (COI).
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